AMRL-TR-69-45

ELECTRON MICROSCOPIC AND MORPHOMETRIC STUDY OF MONKEY AND DOG LUNGS EXPOSED TO BERYLLIUM-CONTAINING DUST

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AUGUST 1969

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AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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100 - October 1969 - CO455 - 92-2039

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
University of Berne, Berne, Switzerland* and Aerospace Medical Research Laboratory, AMD, AFSC Wright-Patterson Air Force Base, Ohio**	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

ELECTRON MICROSCOPIC AND MORPHOMETRIC STUDY OF MONKEY AND DOG LUNGS EXPOSED TO BERYLLIUM-CONTAINING DUST

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report, November 1967-December 1968

5. AUTHOR(S) (First name, middle initial, last name)

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6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
August 1969	17	20

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
F61(052)-68-C-0030	AMRL-TR-69-45
b. PROJECT NO. 6302	
c. Task No. 630206	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. Work Unit No. 630206016	

10. DISTRIBUTION STATEMENT

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Aerospace Medical Research Laboratory, Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, OH 45433

13. ABSTRACT

Five monkeys and six dogs were exposed three times for 30 minutes each at monthly intervals to an atmopshere contaminated by a beryllium compound whose chief component was beryllium oxide. Two years after the exposure, the lungs of both test groups, as well as those of two control monkeys and two control dogs, were fixed and prepared for electron microscopic and morphometric analysis. Histologic examination revealed no fibrotic changes of pulmonary parenchyma in the exposed animals. The ultrastructure of lung tissue of exposed animals was identical to that of control animals in every respect. Neither the arithmetic nor the harmonic mean thickness of the air-blood-barrier were changed in the test animals; in particular it was not possible to demonstrate edematous or proliferative changes. It is concluded from the electron microscopic and morphometric examination of these lungs, that the beryllium compound investigated, essentially beryllium oxide, has not caused any pathological alterations in lung tissue two years after exposure.

DD FORM 1 NOV 65 1473

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Beryllium						
Lung Pathology						
Toxicology						
Morphometry						
Electron microscopy						
Monkey Lungs						
Dog Lungs						

Foreword

This work was sponsored by Toxic Hazards Division of the Aerospace Medical Research Laboratory under contract F61 (052)-68-C-0030 with the University of Berne, Berne, Switzerland. The contract was administered through the European Office of Aerospace Research, United States Air Force. The work was performed in support of project 6302, Toxic Hazards of Propellants and Materials; task 630206, Toxicological Support; work unit 630206016, Pulmonary Pathology Caused by Inhaled Beryllium Compounds. Captain Roger L. Sopher, USAF, MC, of the Pathology Branch was the technical monitor for Toxic Hazards Division. When he retired from the service, the work was assumed by Major Paul N. Monteleone, Jr., USAF, MC. This contract effort was initiated in November 1967 and was completed in December 1968.

This technical report has been reviewed and is approved.

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Section I

INTRODUCTION

Among the various manifestations of pneumoconiosis, attention has recently been called to lung changes induced by the inhalation of beryllium compounds of various composition, which are found in industrial dust and are suspected to be a possible contaminant of space cabin atmosphere. This prompted a careful analysis of possible pathogenetic effects on the lung.

Pulmonary pathology due to beryllium (berylliosis) has been repeatedly reported in the literature (Meyer, 1942; Van Ordstrand et al., 1964; Bruce et al., 1950). More recent experimental studies on animals (Spencer et al., 1967; Robinson and Schaffner, 1968; Witschi, 1968; Vacher and Stoner, 1968) have revealed that beryllium compounds can cause pathological manifestations in lung, liver, and blood. The pulmonary damage induced by beryllium salts has been described as chemical pneumonitis (bronchoalveolitis) or as chronic granulomatosis resembling sarcoidosis (Pugliese et al., 1968; Hardy and Tabershaw, 1946).

The exact mechanism of beryllium effect is not known. A number of physical and chemical properties of this compound have been invoked as triggering factors of beryllium disease (Spencer et al., 1967). Since it has been shown in skin tests that hypersensitivity to beryllium compounds can exist, it has been postulated that the pulmonary changes are also due to an immunologic response (Van Ordstrand, 1966; Curtis, 1951). In an experimental study on animals, Robinson and Schaffner (1968) found pulmonary lesions that they interpreted as typical foreign body reactions rather than as lesions of the immunological type. They have reported on results from animals exposed to a mixture containing beryllium oxide, beryllium fluoride, and beryllium chloride.

It appeared important to study with particular care the fine structural and cytological changes induced in lungs by the inhalation of an atmosphere containing a significant amount of beryllium oxide, eliminating as far as possible the other beryllium salts. To account for the chronic type of lung reaction the animals were studied two years after exposure to the contaminated atmosphere.

Section II

MATERIAL AND METHODS

Five adult monkeys and six adult dogs of both sexes were exposed three times to an atmosphere contaminated by increasing concentrations of a beryllium compound designated as Sample 24 (table I). These exposures were carried out in closed chambers under another contract sponsored by the Aerospace Medical Research Laboratory. The average contamination of the atmosphere by beryllium varied between 3.30 and 4.38 mg Be/m^3. Beryllium oxide was the chief component of the sample (No. 24) introduced into the chamber atmosphere. Two monkeys and two dogs were kept as controls.

TABLE I

COMPOSITION OF SAMPLE 24
(from H. C. Spencer, Progress Report)

BERYLLIUM, OXYGEN, CHLORINE, CARBON, AND HYDROGEN IN SAMPLE 24

Element	*Percent (%) of Element in Total Sample*
Be (except Be as $BeCl_2$)	35.3
C	~0.5
H	0.06
Cl	1.21
O	65.7±1.5

X-RAY POWDER DIFFRACTION ANALYSIS OF SAMPLE 24

Components in Total Sample

BeO – Chief component
Fe_2O_3 – ~5%
Nacl – <1%

Two years after exposure the animals were shipped to Wright-Patterson Air Force Base, Ohio, where they were anesthetized with sodium phenobarbital. The dogs received an additional intramuscular injection of 3 mg Tubocurarine chloride. The lungs were immediately fixed in situ by instillation of a glutaraldehyde solution (1.5% in s-collidine buffer, pH 7.4, osmolarity 336 milliosmols) into the airways. The lungs were then shipped to the University of Berne, Switzerland, for further processing in the Department of Anatomy. The lung tissue blocks were post-fixed in collidine buffered osmium-tetroxide and stained en bloc with buffered uranyl-acetate. After dehydration in ethanol the tissue was embedded in Epon 812. Sections were obtained on an LKB Ultrotome III. Section contrast was enhanced with lead citrate. Electron micrographs were recorded in a Philips EM 200 electron microscope.

For the morphometric analysis five sections of each animal were randomly chosen; from each, seven random fields were recorded on 35 mm film. The procedure for selecting random fields and for stereologic analysis of the micrograph have been presented in previous reports (Weibel, Kistler, Scherle, 1966; Kistler, Caldwell and Weibel, 1965). The primary data were recorded by an automatic recording device, from which they were transferred directly to punch cards for evaluation in a computer (Weibel, 1967).

Section III
RESULTS

HISTOLOGY AND ULTRASTRUCTURE OF LUNG TISSUE

Structure and composition of lung tissue of both control and test animals were normal in every respect, with the exception of one control monkey whose lung had aggregates of histiocytes around middle-sized blood vessels. These histiocytes contained birefringent crystalline material of unknown composition. Compared with rat lungs the tissue elements of the intraalveolar septa appear more pronounced. In particular there is a larger number of collagen fibrils distributed throughout the interstitium; occasionally, these form thick bundles (figs. 2 and 6). However, no differences were noted between control and test animals in this respect. The alveolar capillaries are continuously lined by endothelial cells and contain mainly blood plasma, erythrocytes, and occasional white blood cells. The alveoli appear clean without any trace of hemorrhage or intra-alveolar fibrinous exudate. The alveolar epithelial cells, particularly the granular pneumocyte did not show any alterations in the test animals (figs. 3 and 4). The larger structures, bronchioles, arteries and veins, were normal in all cases.

The dog lungs were found to contain a significant number of peculiar cells located in the interstitial space and often had close relations to blood vessels (figs. 7 and 8). They were characterized by an apparently shrunken nucleus with dense chromatin and by a large number of empty membrane-bounded vesicles measuring approximately 0.1 μ in diameter. The small mitochondria of these cells were predominantly located near the nucleus. Many of these cells showed a complicated surface texture with numerous cytoplasmic protrusions. These cells were interpreted as degranulated mast cells by light microscopy, no metachromatic granules could be demonstated by staining with toluidine-blue or thionin.

Mast cells are known to occur in normal dog lungs (Holmgren and Wilander, 1937; Arvy and Quivy, 1955). The frequency of these cells did not differ between control and test animals. Degranulation of mast cells can be induced by fixation with formaldehyde (Hill, 1965); it is therefore possible that glutaraldehyde will have a similar effect. Rilley (1953), and Fulton and West (1965) demonstrated experimentally in rats that administration of d-Tubocurarine chloride causes degranulation of mast cells. The dogs investigated in our study were treated with Tubocurarine prior to sacrifice, consequently this medication may have been another cause of degranulation.

MORPHOMETRIC FINDINGS

The relevant morphometric findings obtained on the present series of dog and monkey lungs are summarized in table II and in figures 10 and 11. In general, the data obtained in test animals did not show any striking differences to the control values. The arithmetic mean thickness of the air-blood-barrier of the test monkeys appears to be somewhat smaller than the control values. This appears to be due to a larger interstitial volume of the control monkeys (fig. 10a). It is not possible to decide whether this difference is statistically significant, since only two control animals were available for study. A comparison with previous data is not permissible since it appears from experience that variation in interstitial volume within a certain range may be due to varying conditions, such as duration of anaesthesia, etc. The difference is however slight and should be regarded as circumstantial, particularly since no such difference is found in the dog lungs (fig. 10b).

In both monkey and dog lungs the harmonic mean of the air-blood-barrier thickness shows no substantial variation (fig. 10). The relationship between capillary and alveolar surface area is linear in both groups and shows no appreciable deviations between test and control groups (fig. 11).

TABLE II

SYNOPSIS OF MORPHOMETRIC DATA

TEST ANIMALS	Weight	Lung Volume	SURFACE AREA		ARITHMETIC MEAN THICKNESS				HARMONIC MEAN
			Alveoli	Capillary	Barrier	Epithelium	Endothelium	Interstitium	Barrier Thickness
			S_A	S_C	$\overline{\tau}$	$\overline{\tau}_{ep}$	$\overline{\tau}_{en}$	$\overline{\tau}_{in}$	τ_h
	kg	ml	$_m2$	$_m2$	μ	μ	μ	μ	μ
CONTROL MONKEYS									
1	–	193	16.21	15.44	1.62	0.25	0.25	0.50	0.52
2	2.15	204	13.46	12.03	1.65	0.25	0.31	0.43	0.53
TEST MONKEYS									
1	3	216	13.72	11.88	1.39	0.28	0.32	0.39	0.46
2	3.95	193	13.89	11.96	1.35	0.38	0.33	0.38	0.45
3	5.75	216	14.90	13.75	1.54	0.34	0.32	0.33	0.49
4	–	206	17.09	16.06	1.57	0.36	0.23	0.39	0.52
CONTROL DOGS									
1	11	460	32.20	32.06	1.42	0.31	0.25	0.42	0.46
2	11	464	33.87	30.62	1.20	0.34	0.21	0.43	0.43
TEST DOGS									
1	12	477	40.06	35.29	1.54	0.33	0.23	0.42	0.49
2	12	350	30.45	25.20	1.35	0.36	0.25	0.37	0.43
3	9	459	33.50	27.45	1.46	0.34	0.21	0.44	0.48
4	10	464	24.18	21.88	1.20	0.30	0.25	0.44	0.42

Figure 1. Electron micrograph of control monkey's lung. Air-blood-barrier contains interstitial elements and cell bodies of endothelial and epithelial cells. X 16,800.

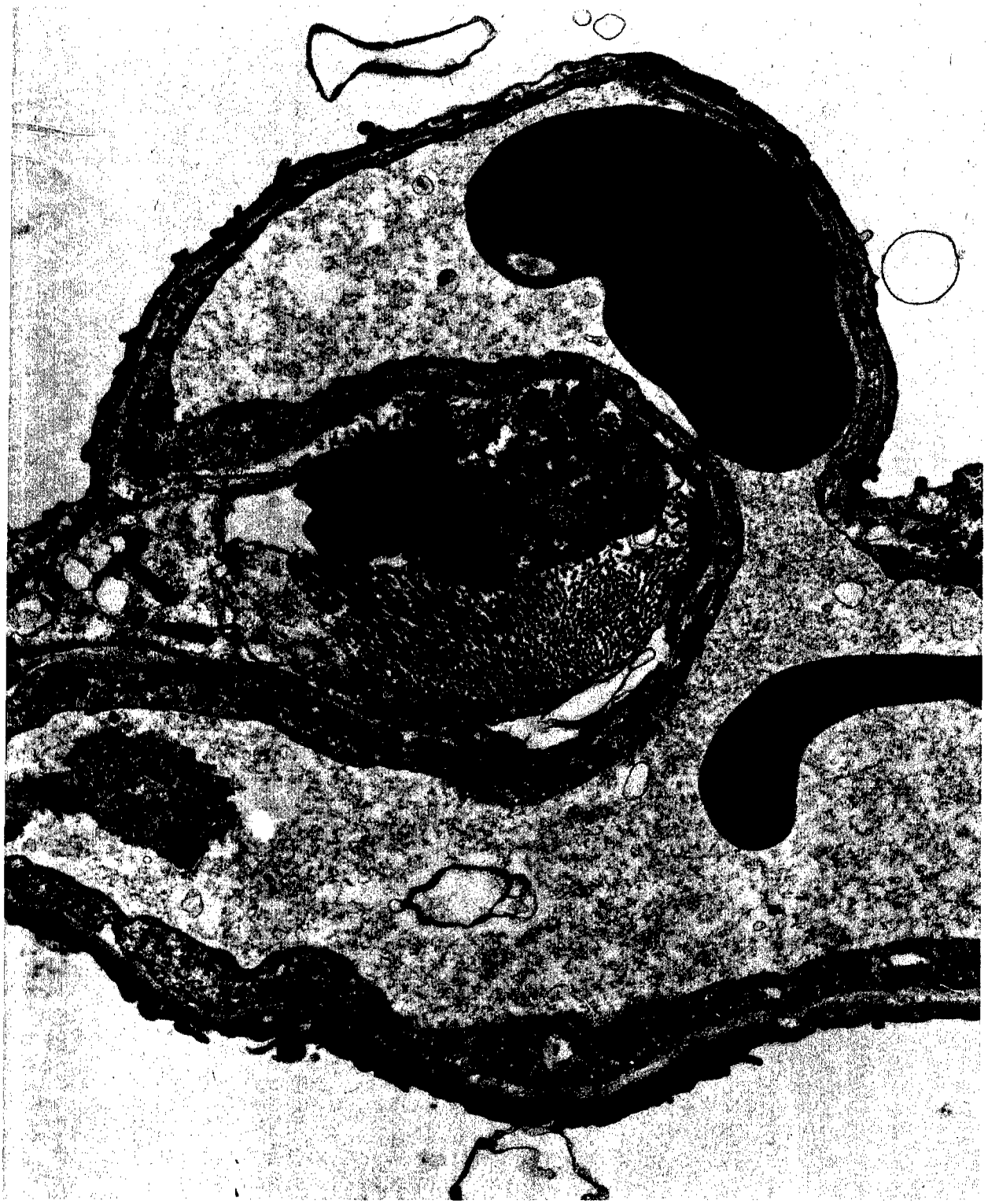

Figure 2. Interalveolar septum of test monkey's lung. Alveolar epithelium and endothelium unchanged. X 21,850.

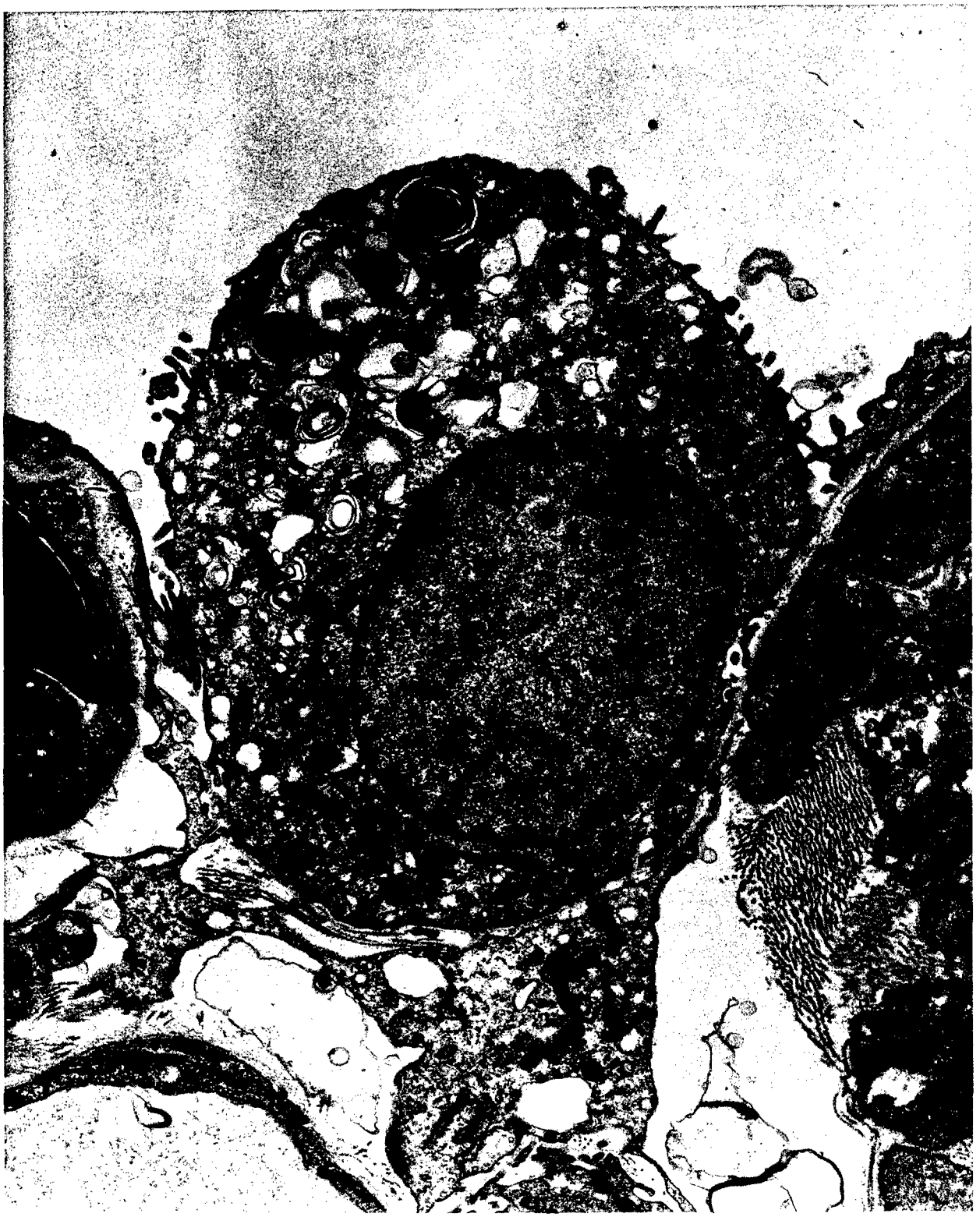

Figure 3. Lung of control monkey. Granular pneumocyte containing lamellated bodies. X 13,250.

Figure 4. Monkey lung exposed to beryllium. Granular pneumocyte with unaltered lamellated bodies. The lung is quite similar to control lung tissue. X 10,300.

Figure 5. Air-blood-barrier of control dog lung with narrow interstitium. X 5,750.

Figure 6. Alveolar capillary of test dog lung. Alveolar and capillary linings unchanged, larger compact collagen bundles in interstitium also occur in control dog lungs. X 21,850.

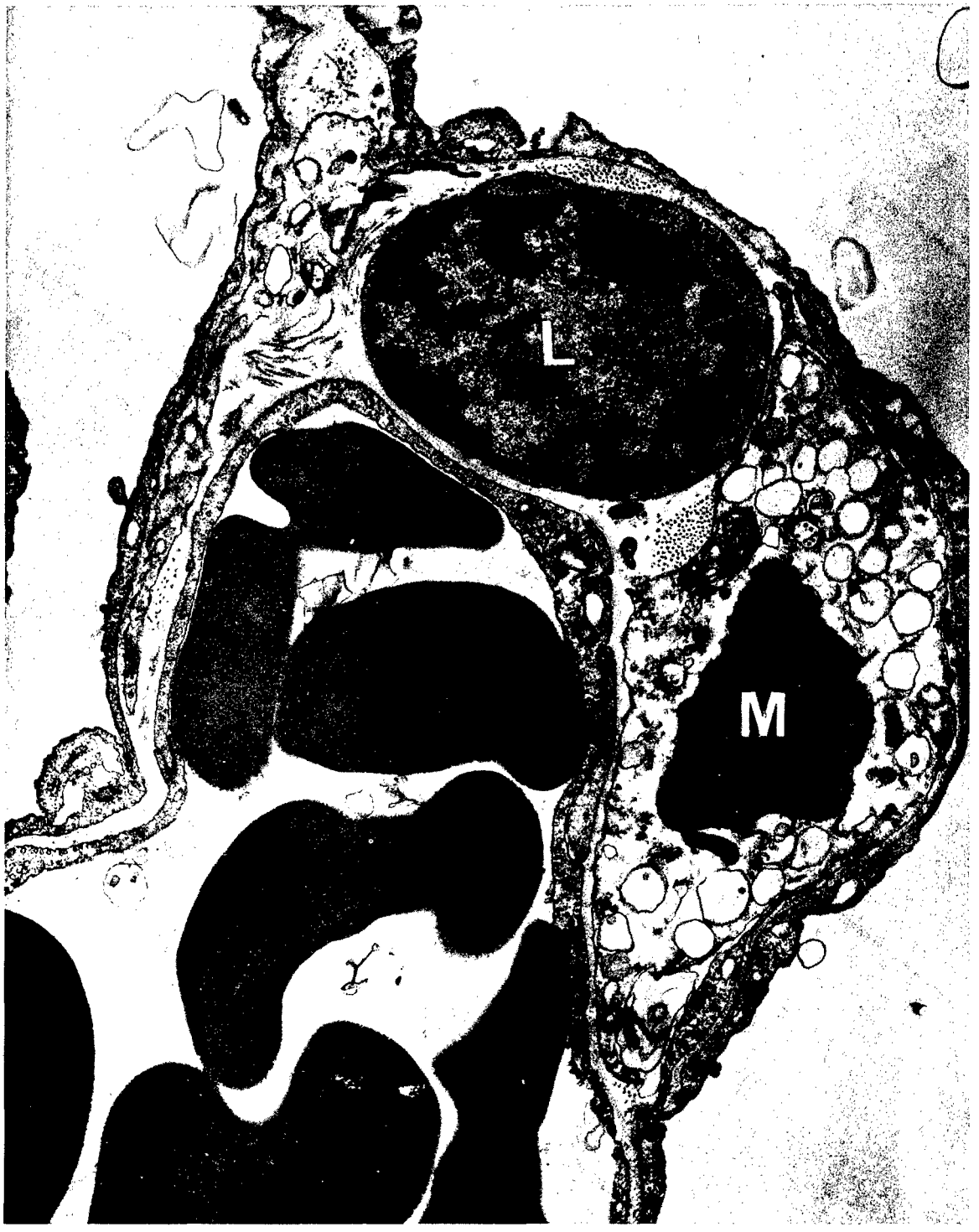

Figure 7. Lung of control dog. Mast cell (M) and lymphocyte (L) in interstitial space in close relation to blood vessels. X 13,250

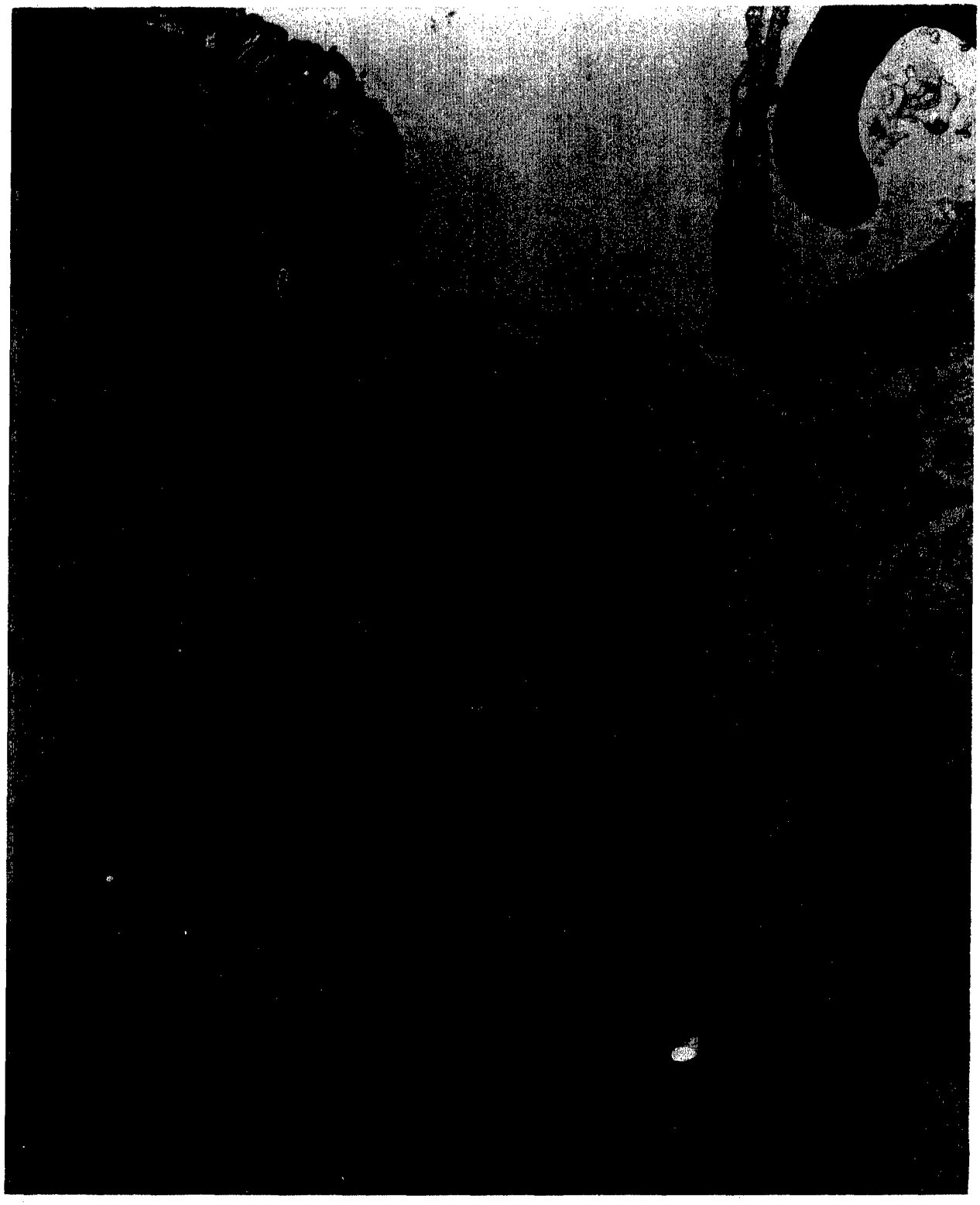

Figure 8. Dog lung exposed to beryllium. Mast cell in relation to blood vessel. Note a few bulky microvilli on the surface, and a honeycomb-like structure of the cytoplasm. X 10,300.

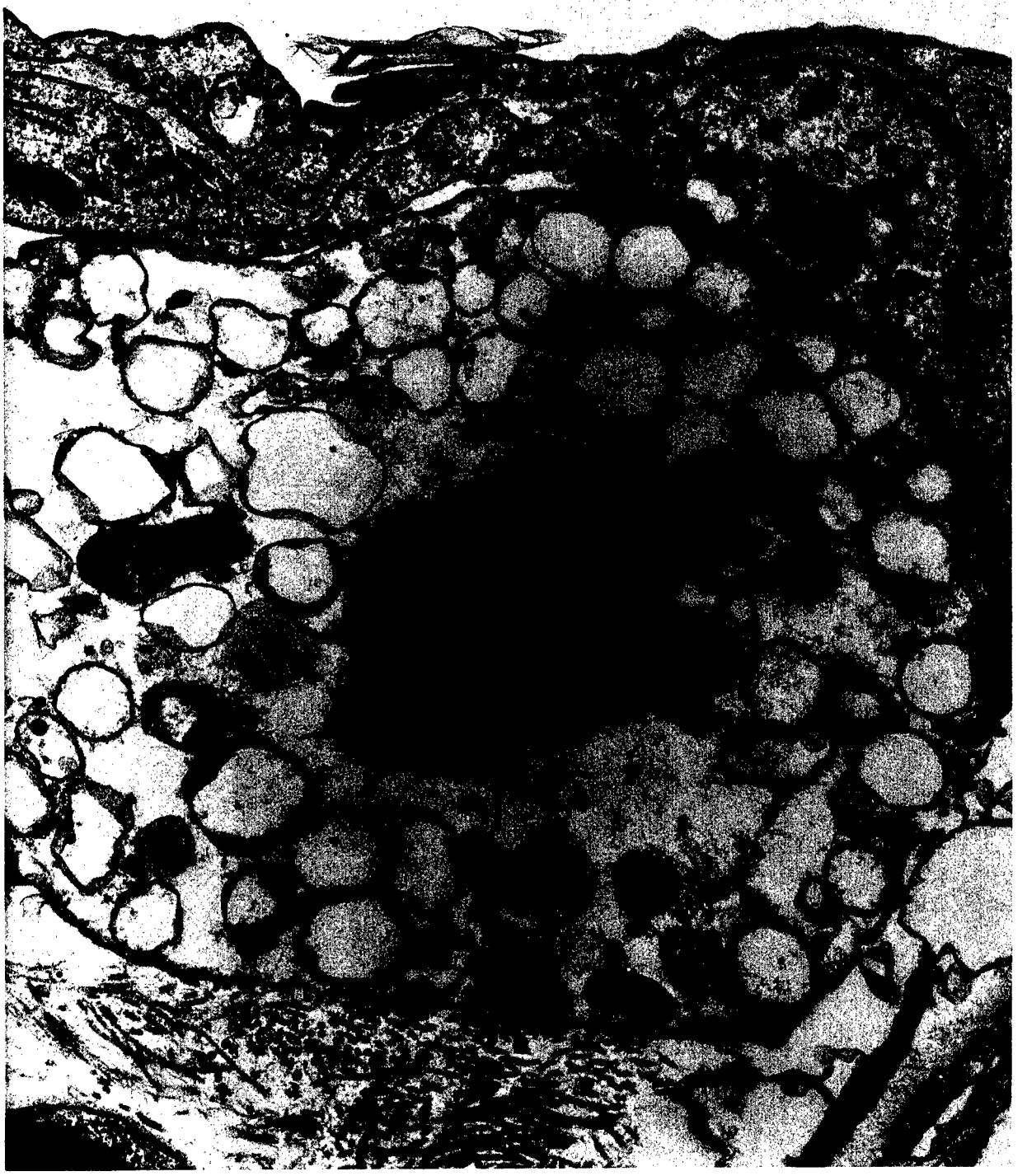

Figure 9. Dog lung exposed to beryllium. Higher magnification of mast cell. X 13,000.

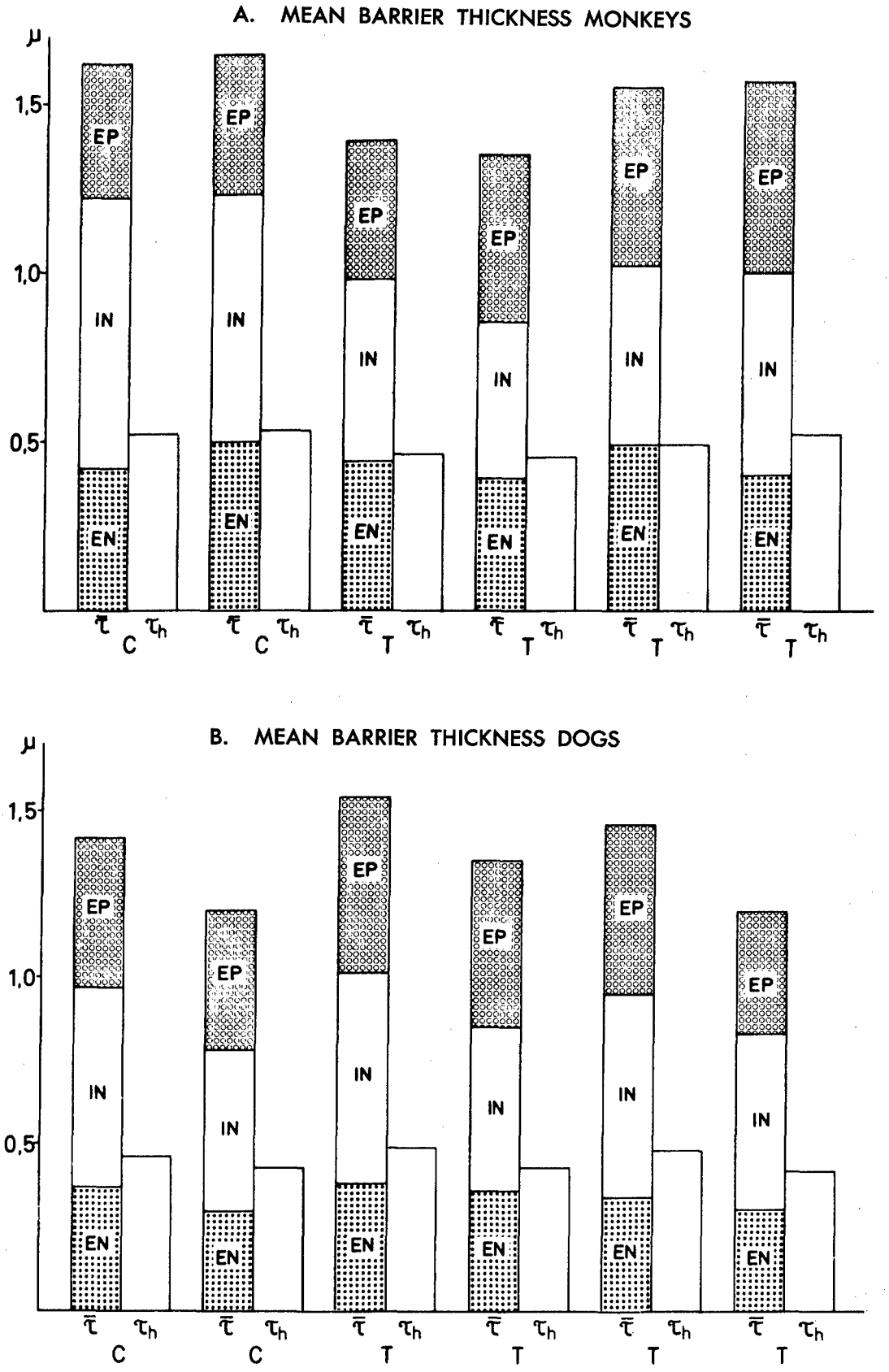

Figure 10. Arithmetic ($\bar{\tau}$) and harmonic (τ_h) mean barrier thickness for control (C) and test (T) monkeys and dogs.

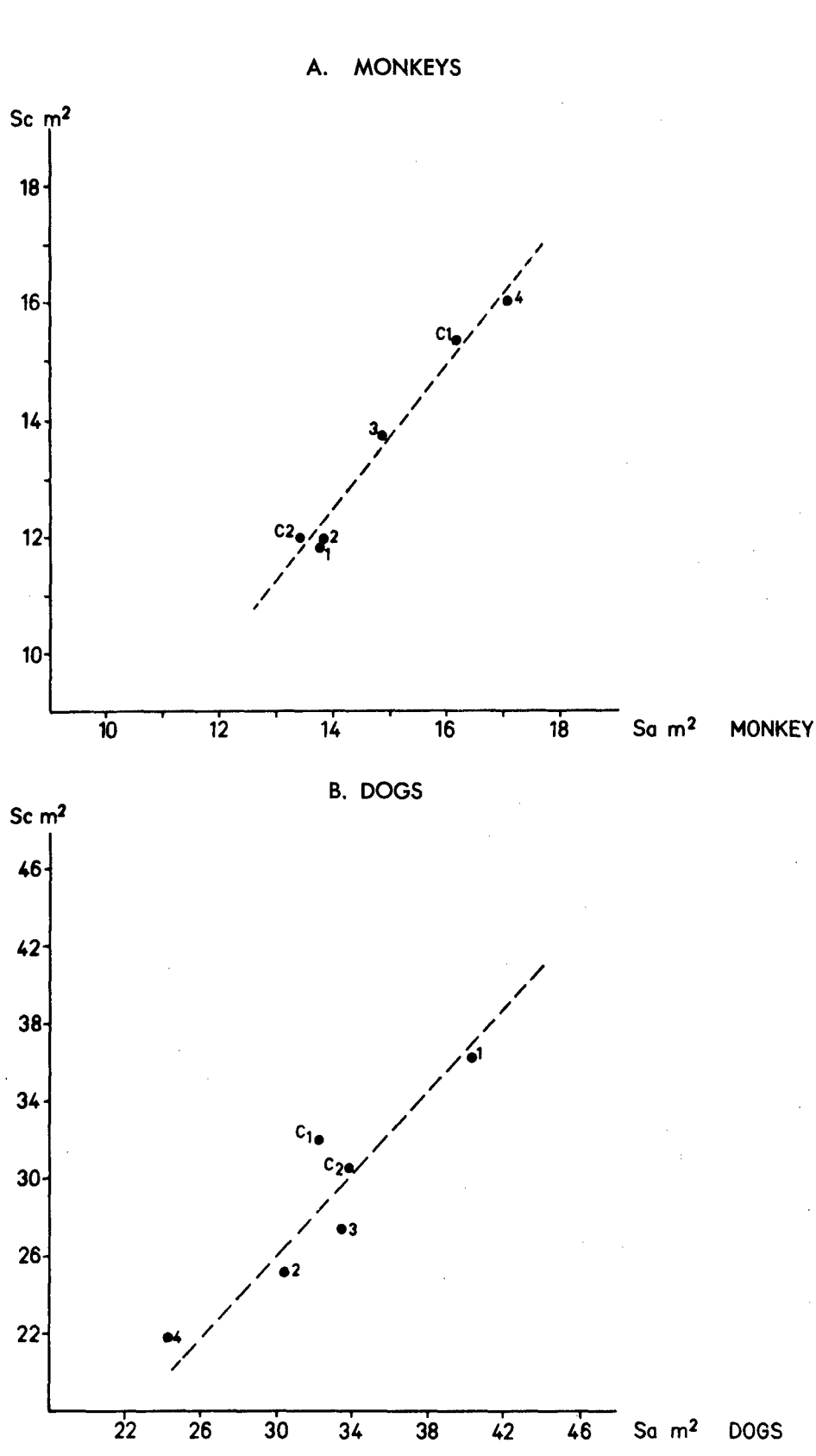

Figure 11. Relationship between alveolar surface area (Sa) and capillary surface area (Sc) in control (C_1-C_2) and test (1, 2, 3, 4) monkeys and dogs.

Section IV
DISCUSSION

The investigation of lungs of monkeys and dogs exposed to an atmosphere contaminated by beryllium oxide 2 years before sacrifice revealed no pathological changes in lung structure at the level of alveoli and capillaries. It has not been possible to demonstrate by either electron microscopy or by light microscopy in polarized light depositions of beryllium compounds. Nor have we been able to detect modifications in the structure of any cell types.

The mast cells found in great number in the dog lungs proved to be normal constituents (Holmgren and Wilander, 1937; Arvy and Quivy, 1957). Although mast cells are known to occur in greater number in association with chronic lung diseases, such as tuberculoma, syphiloma, and lung fibrosis (Arvy, 1955) it can be positively said that they represent no pathological finding in this study, since they were also found in the lungs of control dogs. Furthermore, no signs of chronic proliferative lung changes were found.

Morphometry did not reveal any thickening of the air-blood-barrier. In particular, it was not possible to demonstrate any edematous or proliferative changes in either interstitium, endothelium or epithelium. The tissue mass, as measured by the arithmetic mean thickness of the air-blood-barrier, was not increased; the unchanged harmonic mean thickness indicates that the diffusion resistance of the air-blood-barrier is unchanged.

Robinson and Schaffner (1968) investigated animals exposed to an atmosphere containing mixed beryllium compounds with beryllium oxide as the main contaminant. They observed that a majority of the animals, but not all, developed chronic lung disease, which they interpreted as foreign body reaction combined with proliferative changes. Consequently the results of that study contrast with those presented in this report. Two alternative explanations can be presented: Spencer et al. (1967) have shown that different physical and chemical properties of beryllium oxide will also result in different degrees of toxicity. The inhalation of beryllium oxide calcinated at 500C over a period of 10 hours can lead to severe lung damage, even inducing the formation of adenocarcinoma. However, only very mild lesions are observed after the inhalation of beryllium oxide calcinated between 1100 and 1600C. The compound used in the present study was calcinated at 1400C, while nothing is known about calcination of the compound used in Robinson and Schaffner's study (1968). Robinson and Schaffner reported results in animals exposed to a mixture of beryllium compounds containing 50% beryllium oxide, 40% beryllium fluoride and some beryllium chloride. They observed that beryllium fluoride not only accumulated in the lung, causing beryllium deposits and severe local damage, but that it was also carried into the entire body by the blood, where it led to severe alterations in skeleton, liver, spleen, and kidneys. It is therefore quite possible that the pulmonary damage was not caused by beryllium oxide but by the much more toxic beryllium fluoride.

On the basis of the results presented in this report it can be concluded that the inhalation of beryllium oxide under the prevailing experimental conditions did not induce any detectable pathological changes in lung structure within a period of 2 years after exposure.

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